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Bidirectional Neuro-Glial Signaling Modalities in the Hypothalamus: Role in Neurohumoral Regulation

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Abstract

Maintenance of bodily homeostasis requires concerted interactions between the *neuroendocrine* and the *autonomic nervous systems*, which generate adaptive neurohumoral outflows in response to a variety of sensory inputs. Moreover, an exacerbated neurohumoral activation is recognized to be a critical component in numerous disease conditions, including hypertension, heart failure, stress, and the metabolic syndrome. Thus, the study of neurohumoral regulation in the brain is of critical physiological and pathological relevance. Most of the work in the field over the last decades has been centered on elucidating *neuronal* mechanisms and pathways involved in neurohumoral control. More recently however, it has become increasingly clear that non-neuronal cell types, particularly astrocytes and microglial cells, actively participate in information processing in areas of the brain involved in neuroendocrine and autonomic control. Thus, in this work, we review recent advances in our understanding of neuro-glial interactions within the hypothalamic supraoptic and paraventricular nuclei, and their impact on neurohumoral integration in these nuclei. Major topics reviewed include anatomical and functional properties of the neuro-glial microenvironment, neuron-to-astrocyte signaling, gliotransmitters, and astrocytes regulation of signaling molecules in the extracellular space. We aimed in this review to highlight the importance of neuro-glial bidirectional interactions in information processing within major hypothalamic networks involved in neurohumoral integration.

Keywords

paraventricular; supraoptic; astrocytes; microglia; sympathetic; neuroendocrine; oxytocin; vasopressin; nitric oxide; glutamate; GABA

1- Introduction

Maintenance of bodily homeostasis requires concerted interactions between the *neuroendocrine* and the *autonomic nervous systems*. By controlling these two major information processing systems, specific neuronal networks within the central nervous system (CNS) generate adaptive *neurohumoral* responses, which are necessary for proper cardiovascular, fluid and energy balance system regulation. Neurohumoral activation is not only important within the context of adaptive physiological responses, but it is now also recognized to be a critical pathophysiological component in numerous disease conditions,

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including hypertension, heart failure, stress, and the metabolic syndrome (Brook et al., 2000; Cohn et al., 1981; Felder et al., 2001; Haywood et al., 1985; Middlekauff et al., 1998; Perez-Tilve et al., 2006). In the case of heart failure, for example, a compelling correlation between neurohumoral activation, morbidity and mortality in heart failure patients has been established (Cohn et al., 1984). Thus, elucidating precise anatomical pathways and cellular mechanisms underlying the generation of neurohumoral outflows by the brain is of critical physiological and clinical relevance.

Within the central neuronal circuitry involved in autonomic and neuroendocrine regulation, the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei stand as pivotal centers involved in the generation of coordinated neurohumoral responses (Cohn et al., 1984) (Guyenet, 2006; Swanson et al., 1980a; Swanson et al., 1983). Neurons in the PVN and SON are activated in response to a variety of afferent stimuli triggered by disturbances in the internal milieu, including dehydration, changes in blood pressure and blood volume, among others (Badoer et al., 1997; Krukoff et al., 1997; Li et al., 1994; Lovick et al., 1988; McKinley et al., 1994; Randolph et al., 1998; Schiltz et al., 1997; Stocker et al., 2006). These afferent stimuli elicit in turn robust autonomic and neuroendocrine responses coordinated by tese nuclei, which include increases in sympathetic outflow to the kidney and vasopressin release (Stocker et al., 2005; Stocker et al., 2004; Stricker et al., 2002).

The PVN is a highly heterogeneous nucleus, containing functionally diverse groups of neurons (Swanson et al., 1983). These include: a) magnocellular neurosecretory cell (MNCs), which synthesize the neuropeptides vasopressin (VP) and oxytocin (OT). These neurons project to the posterior pituitary, from where VP and OT are released in response to a variety of stimuli, including hyperosmolarity and lactation; b*)* parvocellular neuroendocrine neurons, which project to the median eminence and secrete hypophysiotropic hormones, including corticotropin-releasing factor, thyrotropin-releasing hormone, and somatostatin; and c) parvocellular preautonomic neurons, which send long descending projections to autonomic centers in the brainstem and spinal cord, and participate in autonomic control (Saper et al., 1976; Swanson et al., 1980b; van den Pol, 1982). The SON on the other hand, is a “purely” neurosecretory center, containing only OT and VP MNCs.

Neurohumoral outflow from these centers is directly dependent on the degree and pattern of neuronal activity of both neurosecretory and preautonomic neurons (Akine et al., 2003; Allen, 2002; Cazalis et al., 1985; Poulain et al., 1982; Stocker et al., 2004). Thus, elucidating precise mechanisms that regulate firing activity in these centers has been a major focus of work in this field. Over the last two decades, we have gain significant insights on intrinsic membrane properties and synaptic physiology of magnocellular and preautonomic PVN/SON neurons, and we refer the readers to excellent reviews and articles on this topic (Akine et al., 2003; Armstrong et al., 1998; Bourque, 2008; Brown, 2004; Chen et al., 2009; Iremonger et al., 2008; Luther et al., 2000; Sonner et al., 2007; Stern, 2001). More recently however, it has become increasingly clear that non-neuronal cell types, particularly astrocytes and microglial cells, actively participate in information processing in the brain (Araque et al., 2010; Perea et al., 2009; Volterra et al., 2005). Thus, the focus of the present review will be on recent advances in our understanding of neuro-glial interactions within the hypothalamic SON/PVN, with particular emphasis on astrocytes, and to a lesser extent microglial cells, and their impact on neurohumoral integration by these nuclei.

2- Bidirectional neuro-glia communication in the brain

While for many years astrocytes were regarded as passive supportive cells, the use of Ca^{2+} imaging technology (Tsien, 1980; Tsien, 1981) unraveled their contribution in numerous

processes including: development (Rakic, 2003), synaptic transmission (Perea et al., 2010; Volterra et al., 2005), modulation of neuronal networks (Araque et al., 2010; Giaume et al., 2010) and the control of cerebral blood flow (Attwell et al., 2010; Filosa et al., 2006; Mulligan et al., 2004; Takano et al., 2006; Zonta et al., 2003), to name a few. The finding that astrocytes express a plethora of receptors and respond to numerous neurotransmitters gave name to the tripartite synapse model (Araque et al., 1999), which defines the ability of astrocytes not only to listen to neurons, but also to talk back. Astrocytes are equipped with the machinery to release gliotransmitters (Parpura et al., 2010; Parpura et al., 1994) (e.g. glutamate, D-serine, ATP), and also express specialized transporters, which aid in the modulation of synaptic activity by altering the milieu at the synaptic/peri-synaptic space. Moreover, astrocytes play an important role in the buffering of extracellular glutamate and K^+ (Walz, 2000). Anatomically, a single astrocyte can enwrap thousands of synapses and create a physical barrier allowing direct interaction with neurons as well as neural networks (Giaume et al., 2010). Although controversial (Agulhon et al., 2008), a role for astrocytes in synaptic plasticity has been demonstrated, including both long term potentiation (LTP) and long term depression (LTD) (Henneberger et al.; Panatier et al., 2006a; Pascual et al., 2005; Perea et al., 2007; Zhang et al., 2008). For example, astrocytes in the SON, via the release of the aminoacid D-serine (see more below) modulates the efficacy of NMDA-mediated long-term synaptic plasticity in MNNs) (Panatier et al., 2006b).

Importantly, numerous developments of our current understanding of neuro-glial interactions in the brain emerged from pioneering work in hypothalamic centers involved in neurosecretory and autonomic control, including the SON and PVN. This has been the topic of excellent previous reviews (Hatton, 2004; Oliet et al., 2008b; Tasker et al., 2012; Theodosis et al., 2008), and an account of some of the most salient and recent developments in this area are summarized and discussed here.

3- Unique anatomical characteristics of the neuro-glial microenvironment in the SON and PVN

When considering functional interactions among neurons and glial cells in the SON/PVN, it is important to take into consideration several distinctive aspects of these centers. For example, detailed anatomical studies obtained from the magnocellular neurosecretory system in the SON/PVN revealed a highly “compact” neuro-glial microenvironment, in which the surface membrane of principal neurons are largely surrounded by astrocyte processes. Thus, under normal physiological conditions, astrocytes act as a physical and chemical barrier, limiting neuron-neuron interactions, as well as the diffusion of neurotransmitters in the extracellular space. Remarkably however, during conditions known to stimulate SON/PVN neuronal activity, such as dehydration and lactation, in the case of VP and OT neurons, respectively, the neuro-glial microenvironment undergoes a dramatic structural remodeling (Hatton, 2004; Oliet et al., 2008a; Piet et al., 2004; Tasker et al., 2012; Theodosis et al., 1989; Theodosis et al., 1999). This plasticity involves rapid and reversible glial retraction from between neighbouring neurons, unwrapping their synaptic contacts as well. This activity-dependent structural remodelling results in increased neuron-to-neuron juxtapositions, accumulation of extracellular K^+, and build up of neurotransmitter levels in the extracellular space (see more below), all of which facilitate homeostatic responses in order to properly cope with these physiological challenges. A caveat to be taken into consideration, however, is that most of these studies relate to the magnocellular neurosecretory system. Thus, whether a similar neuro-glial organization and plasticity can be extrapolated to other neuronal types in the PVN, particularly preautonomic neurons, is at present unknown. Nonetheless, the SON/PVN have already proven to be a unique experimental model to investigate neuro-glial interactions in the brain.

4- Neuron-to-Astrocyte signaling

In addition to classical neurotransmitters, such as glutamate and GABA (Decavel et al., 1990; van den Pol et al., 1990), an abundance of functionally-relevant neuropeptides, including VP, OT, a-MSH, NPY, AGRP, dynorphin, galanin and endothelins, among others (Hokfelt et al., 2000; Landgraf et al., 2004; Ludwig et al., 2006) are pivotal signalling molecules within hypothalamic neuronal networks.

Here, we summarize recent studies supporting communication from neurons to astrocytes in the SON and PVN, via classical neurotransmitters as well as neuropeptides.

Noradrenaline and glutamate

In an elegant study, Gordon et al (2005) showed that noradrenaline binds on α_1-adrenoceptors on PVN astrocytes to stimulate ATP release (Gordon et al., 2005), which then acting on neighbouring MNCs, leads to the Ca^{2+}-dependent insertion of AMPA receptors, increasing neuronal sensitivity to synaptically-released glutamate. In another study, the same group showed that activation of mGLUR1,5 receptors by synaptically released glutamate was also capable of evoking ATP release from PVN astrocytes (Gordon et al., 2009). Along these lines, Espallergues et al. showed that NE and ATP acted synergistically to increase $[Ca^{2+}]i$ on SON astrocytes. However, the functional consequences of the evoked changes in astrocytes $[Ca^{2+}]i$ were not further assessed (Espallergues et al., 2007).

Results from these experiments provide noteworthy insights into the potential contribution of neuro-glial communication to the generation of integrative neurohumoral homeostatic responses by the PVN. Catecholaminergic afferent inputs originating from A1 caudal medulla neurons and from A2 neurons in the nucleus of the tractus solitarious (Cunningham et al., 2004; Renaud et al., 1991), provide critical visceroceptive afferent inputs carrying information about blood volume and pressure to the SON and PVN nuclei. On the other hand, glutamatergic inputs arising from circumventricular organs provide osmosensitive inputs to these nuclei (Oldfield et al., 1991; Richard et al., 1995; Sladek et al., 1998). Thus, distinct sensory modalities are conveyed to the SON/PVN via neuroanatomically and neurochemically separate neuronal pathways. Within this context, the studies summarized above suggest that SON and PVN astrocytes may act as “bridges” between catecholaminergic and glutamatergic afferent inputs, contributing therefore to the integration of distinct afferent input modalities, a necessary feature for the generation of concerted homeostatic responses.

Endothelins

In a recent study, we focused on a family of peptides abundantly present in the hypothalamus, the endothelins (ETs) (Filosa et al., 2012). ETs are composed of three isoforms, ET-1, ET-2 and ET-3, and their biological actions are mediated by two well characterized G-protein-coupled receptors: ET_A, which exhibits higher affinity for ET-1 than for ET-2 and ET-3, and ET_B, which displays similar affinity for all three isoforms (Davenport, 2002; Watts, 2010; Yanagisawa et al., 1988). While ETs are ubiquitously produced by endothelial cells, neurons (including MNCs), as well as astrocytes, also synthesize ETs (Ehrenreich et al., 1991; Nakamura et al., 1993; Yanagisawa et al., 1988). A large body of work supports an important role for ETs in the regulation of the SON/PVN neurosecretory and autonomic systems (Rossi, 2004; Rossi et al., 2006; Rossi et al., 2001; Rossi et al., 1997a; Rossi et al., 1997b). For example, activation of ET_B receptors stimulated both somatodendritic, as well as neurohypophyseal VP release (Rossi, 2004). Nonetheless, the precise mechanisms by which ETs influence neuronal activity in the hypothalamus were not explored in detailed. We found that ET_B receptor activation in SON astrocytes induced

mobilization of $[Ca^{2+}]i$, which in turn, via activation of glutamate and nitric oxide signaling pathways, evoked excitatory and inhibitory effects on neuronal firing activity, respectively (see Figure 1) (Filosa et al., 2012). Thus, our studies support the view that ETs participate as signaling molecules mediating neuroglial communication in the SON/PVN. It is important to mention however that in a similar study, Zampronio et al. (Zampronio et al., 2010) did not observe ETs-mediated changes in astrocytes Ca^{2+}, despite the fact that ETs efficiently affected the firing activity of OT and VP neurons.

Oxytocin and vasopressin

In addition to being released from neurohypophyseal terminals in the posterior pituitary, the neuropeptides OT and VP are also released from the somata and dendrites of MNCs within the SON and PVN (Ludwig et al., 2006). Somatodendritic release of these neuropeptides plays a critical role in modulating neurosecretory outflow from the SON and PVN, serving as powerful autocrine/paracrine signals by which neurosecretory neurons autoregulate their own firing activity (Gouzenes et al., 1998; Ludwig et al., 1997). Still, the question as to whether they also participate as important signaling molecules targeting local astrocytes, has been explored to a much lesser extent. For example, OT is essential for the induction of activity-dependent neuroglial remodeling in the SON (Langle et al., 2003; Theodosis et al., 1986). Moreover, OT receptors are expressed in hypothalamic cultured astrocytes (Di Scala-Guenot et al., 1992). However, it is still unknown whether OT-dependent neuroglial remodeling involves direct actions of OT on astrocytes. Thus, future studies evaluating the actions of these two major SON/PVN neuropeptides on astroglial function are warranted.

5- Astrocyte-to-neuron signaling (Gliotransmitters)

In addition to being capable of sensing neurotransmitters released by neighbouring neurons, there is now compelling evidence that astrocytes can also release a variety of neuroactive substances, termed gliotransmitters. These include ATP, adenosine, D-serine, glutamate and TNF-α, among others (Gordon et al., 2005; Gordon et al., 2009; Halassa et al., 2009; Kang et al., 1998; Panatier et al., 2006b; Perea et al., 2010; Stellwagen et al., 2006; Tritsch et al., 2007), now recognized to play an important role in information processing within neurosecretory and autonomic hypothalamic networks. As mentioned already above, ATP is one such functionally relevant gliotransmitters (Gordon et al., 2005). Here we summarize recent work providing evidence in support of D-serine, taurine and nitric oxide as gliotransmitters influencing synaptic physiology and neuronal activity in the SON/PVN.

D-serine

D-serine is an amino acid exclusively released from astrocytes, which serves as an endogenous co-agonist of NMDA receptors (NMDARs) (Mothet et al., 2000). In an elegant study, Panatier et. al. demonstrated that D-serine is produced by SON astrocytes, which when released to the surrounding environment, enhanced the amplitude of NMDA-mediated EPSCs and contributed to long-term synaptic potentiation in non-identified MNCs (Panatier et al., 2006b). Importantly, when the astrocytic coverage of MNCs was reduced in lactating rats, NMDAR activity was impaired due to a deficiency in D-serine (Panatier et al., 2006b). These results support D-serine as a pivotal gliotransmitter influencing excitatory synaptic function in the SON, and highlight the impact that dynamic changes in the local neuro-glial microenvironment have on information processing.

Taurine

Next to glutamate, taurine is the second most abundant amino acid in the brain (Palkovits et al., 1986). Taurine is mainly known for its involvement in cell volume regulation, being one of the major inorganic cellular osmolytes used to compensate for changes in extracellular

osmolarity. Taurine is concentrated intracellularly, and can be released following cell swelling in response to a hypo-osmotic stimulus (Martin et al., 1990; Pasantes-Morales et al., 1990). Taurine is abundantly found in the hypothalamus, where it concentrated in glial cells (Decavel et al., 1995; Palkovits et al., 1986). Importantly, taurine release from SON astrocytes is stimulated with high sensitivity by a local hypo-osmotic stimulus (<5% decrease in osmolarity), and is inhibited by a hyperosmotic stimulus (Deleuze et al., 1998; Hussy et al., 1997). Additionally, taurine is an agonist of extrasynaptically located glycinergic receptors, evoking a robust inhibitory action of SON MNCs (Hussy et al., 1997). Thus, taurine release by astrocytes contributes to inhibition of neurosecretory OT and VP neurons in response to a hypo-osmotic stimulus, playing an important role in the regulation of adaptive neurohumoral responses by the magnocellular neuroendocrine system (Hussy et al., 2000). An important implication of these studies is that modulation of gliotransmitter release from SON/PVN astrocytes is not only dependent on signals arising from neurons (e.g., glutamate, norepinephrine), but can also be directly influenced by functionally-relevant systemic stimuli, such as a change in plasma osmolarity. In other words, and as proposed by Hussy et al., (Hussy et al., 2000), astrocytes may act as direct osmosensors, contributing in turn to osmotic-mediated neurohumoral responses. This notion is further supported by a recent study showing that a systemic hyperosmotic stimulus lead to FOS expression in SON astrocytes, which preceded FOS expression in neurons. Moreover, the hyperosmotic-mediated FOS expression in neurons was prevented by the glial metabolic blocker fluorocitrate (Yuan et al., 2010). Taken together, these results support a major role for astrocytes as mediators of osmotically-driven neurohumoral responses.

Gas molecules: nitric oxide (NO) and carbon monoxide (CO)

The gas molecule nitric oxide (NO) acts as a key signaling molecule within the SON and PVN, playing major roles in neurohumoral regulation. Constitutively produced NO tonically inhibits neurosecretory and preautonomic neuronal activity (Li et al., 2002; Li et al., 2003; Stern, 2004; Stern et al., 2001) restraining in turn sympathohumoral outflow to the circulation (Krukoff, 1999; Zhang et al., 1997). Importantly, blunted NO function within the SON/PVN is linked to neurohumoral activation in heart failure (HF) and diabetes (Zhang et al., 2001; Zheng et al., 2005). It is generally assumed that constitutive NO in the SON/PVN arises from a neuronal (nNOS) source. However, an alternative source of constitutive NO in the brain is the endothelial isoform (eNOS), commonly found to be expressed both in endothelial cells and astrocytes (Doyle et al., 1997; Iwase et al., 2000; Lin et al., 2007; Topel et al., 1998). In a recent study, we demonstrated high expression of eNOS within the SON and PVN, which was largely confined to astrocytes (Biancardi et al., 2011). Moreover, using a combination of fluorescent imaging, patch-clamp electrophysiology, along with in vivo sympathetic nerve recordings, we showed that astroglial eNOS contributes to NO bioavailability, as well as tonic inhibition of presympathetic neuronal activity and sympathoexcitatory outflow from the PVN (Biancardi et al., 2011) (Figure.2). Finally, we found astrocyte eNOS expression and function to be blunted in the PVN of heart failure rats. Taken together, these results support NO as another major gliotransmitter in the SON/PVN, playing an important role in neurohumoral regulation, both in control and disease conditions.

Another biologically active gas is carbon monoxide (CO) (Maines, 1993; Verma et al., 1993). While CO has been the focus of numerous studies in cerebral vascular biology (Motterlini et al., 2002), a growing body of evidence also supports CO as a gaseous neurotransmitter within the CNS (Maines, 1993; Snyder et al., 1998; Verma et al., 1993). CO is generated endogenously by heme oxygenase (HO), an enzyme that cleaves the hemin derived from heme-proteins (Fe-protoporphyrin) such as hemoglobin, myoglobin and cytochromes, resulting in the formation of CO, free iron and biliverdin (Maines, 1993;

Verma et al., 1993). There are three isoforms of HO: (a) the inducible HO-1, which can be upregulated by a variety of stimuli including oxidative stress and inflammation (Ewing et al., 1991; Verma et al., 1993); (b), the constitutive isoform HO-2, widely expressed in several tissues, and shown to be regulated by a few selected stimuli, including glucocorticoids (Raju et al., 1997); and (c) HO-3, whose function is yet unknown (Raju et al., 1997). HO-1 is expressed in specific CNS regions, including the hypothalamus (Ewing et al., 1992; Vincent et al., 1994). In fact, a growing body of evidence supports a modulatory role for CO on OT and VP release (Gomes et al., 2010; Mancuso et al., 1999). In a recent study, we reported HO1 to be expressed not only on SON/PVN magnocellular neurons, but also in astrocytes within these nuclei (Reis et al., 2012). This is in agreement with previous studies showing HO-1 presence in astrocytes and their ability to contribute to CO bioavailability in other brain regions (Li et al., 2008; Mancuso, 2004; Schipper et al., 2009). Importantly, we found CO to tonically stimulate SON neuronal activity, having thus opposite effects to NO (Reis et al., 2012). Thus, astrocytes in the SON/PVN are important cellular sources of two opposingly acting gas gliotransmitters, NO and CO. Given their unique biological properties as gas molecules, including their ability to readily diffuse, and to freely cross cell membranes, it is likely that NO/CO may affect relatively distant targets (~100-200 μm) from its source of production (Santos et al., 2012; Wood et al., 1994), suggesting that astrocytes in the SON/PVN may lack well-defined spatial domains, compared to astrocytes in other brain regions (Bushong et al., 2002) (see more below).

Astrocyte regulation of concentration, diffusion and half-life of signaling molecules

Another major mechanism by which astrocytes influence neuronal activity within the tripartite synapse, is via regulation of the time course and concentration of neurotransmitters in synaptic and perisynaptic areas. This is achieved largely by the activity of selective and powerful neurotransmitter uptake transporters, particularly for the amino acid transmitters glutamate and GABA. These include the astrocytic GLT1 and GLAST glutamate transporter isoforms, which account for most glutamate clearance in the CNS (Rothstein et al., 1996), as well as the GAT3 isoform of the GABA transporter (Minelli et al., 1996; Ribak et al., 1996). According to the activity of these transporters, as well as their physical proximity to the source of their substrates, astrocytes can influence a variety of physiologically relevant processes, including modulation of heterosynaptic interactions between excitatory and inhibitory synaptic inputs. Synaptically-released glutamate can diffuse beyond its release site (i.e., spillover) (Isaacson, 2000), and activate metabotropic mGLUR receptors expressed in GABAergic terminals, resulting in depression of GABA release. This process, known as heterosynaptic depression, takes place in the SON and PVN (Piet et al., 2004; Schrader et al., 1997), and is tightly regulated by astrocytes. The activity of astrocyte GLT1 transporters efficiently “buffer” perisynaptic glutamate levels, limiting their access to presynaptic mGLURs in GABAergic terminals. However, pharmacological block of GLT1, or the physical retraction of astrocytic processes during lactation or dehydration, results in diminished neurotransmitter clearance, build-up in the extracellular space, and enhanced diffusion capacity. This has several consequences, including facilitation of heterosynaptic interactions via activation of presynaptic mGLURs (Di et al., 2004; Oliet et al., 2001; Piet et al., 2004).

Astrocytes processes and neurotransmitter transporters also play important roles in regulating the ability of extracellular neurotransmitters to access and activate extrasynaptic receptors. Besides acting on conventional postsynaptic receptors, glutamate and GABA can also activate receptors located extrasynapticaly (Dalby et al., 2003; Le Meur et al., 2007; Sah et al., 1989). Extrasynaptic receptors have been typically considered a nonfunctional pool of receptors, which could be recruited to active synapses ‘on-demand’ (Clark et al., 2002; Harney et al., 2008). However, extrasynaptic receptors are recognized to play

important roles in information processing within the CNS, mediating quite a distinct communication modality, when compared to their synaptic counterparts. Synaptic receptors mediate spatially and temporally restricted transfer of information between two neurons (i.e. postsynaptic current; EPSC). On the other hand, given their characteristic low degree of desensitization and high-affinity for their agonists (Monyer et al., 1992; Priestley et al., 1995; Tovar et al., 1999), extrasynaptic receptors mediate a persistent, 'tonic' current, assumed to more globally influence neuronal excitability, as well as the overall gain within a network of neurons. We recently demonstrated the presence of functional extraynaptic NMDARs in SON neurosecretory neurons (Fleming et al., 2011). Their activation by ambient, extracellular glutamate levels results in a persistent inward current, which tonically stimulates SON neuronal activity. As with mGLURs, astrocyte GLT1 transporters determine the efficiency of this alternative excitatory modality. Thus, either pharmacological block of GLT1 activity or glial retraction during dehydration, results in an enhanced activation and contribution of eNMDARs to both oxytocin and vasopressin SON firing activity (Fleming et al., 2011). We proposed this phenomenon to contribute to the homeostatic increase in neurosecretory firing activity during dehydration. In addition, blockade of GLT1 activity within the PVN resulted in an enhanced renal sympathetic nerve activity (Dr. Patel, KP, University of Nebraska, unpublished observations). Finally, we recently reported a blunted GLT1 expression and function in SON neurons in rats with ischemic heart failure, a disease characterized by neurohumoral activation (Potapenko et al., 2012). Thus, astrocyte regulation of extrasynaptic NMDAR-mediating excitatory modality is not only an important signaling mechanism influencing physiological homeostatic neurohumoral responses, but may also contribute to exacerbated neurohumoral activities during disease conditions, such as heart failure.

Similar to the case of glutamate, we also found SON and PVN astrocytes to regulate the ability of extracellular GABA to activate extrasynaptic $GABA_A$ receptors. For example, we reported that blockade of astroglial GABA transporters in the PVN resulted in a buildup of extracellular GABA and activation of extrasynaptic $GABA_A$ receptors in presympathetic PVN neurons, diminishing in turn their ongoing firing activity, as well as sympathoexcitatory outflow to the kidney (Park et al., 2009). Similar results were observed in magnocellular neurosecretory neurons (Park et al., 2006). Taken together, these studies indicate by modulating the degree of extrasynaptic $GABA_A$ receptor activation, astrocytes can efficiently influence the activity of both neurosecretory and preautonomic neurons, affecting consequently neurohumoral outflows from these regions (Park et al., 2009; Park et al., 2007; Park et al., 2006).

Summary and Future directions

We aimed in this review to highlight the importance of neuro-glial bidirectional interactions in information processing in the CNS in general, and within the SON and PVN, major hypothalamic networks involved in neurohumoral integration, in particular. As summarized above, and schematically shown in Figure 3, we provided an overview of several mechanisms and signaling pathways by which neurons and astrocytes communicate with each other in these areas, to ultimately influence the integration of peripheral sensory inputs, as well as the generation of neurosecretory and autonomic outflows from these nuclei. These included the ability of astrocytes to "sense" synaptically-released neurotransmitters (e.g., glutamate, norepinephrine) and to actively release gliotransmitters (e.g., D-serine, ATP, nitric oxide). Moreover, astrocytes are actively involved in the clearance of neurotransmitters from the extracellular space, influencing in turn other important signaling modalities (e.g., heterosynaptic interactions and activation of extrasynaptic receptors). Importantly, these cell-cell communication modalities in these nuclei take place within a unique and remarkable plastic neuro-glial microenvironment.

Despite all the recent advances highlighted in this and other topical reviews (Bains et al., 2007; Hatton, 2004; Oliet et al., 2004; Tasker et al., 2012), there are still essential gaps and unanswered questions in this area of research, which clearly will motivate additional work in the near future. For example, astrocytes have the ability to produce and release a plethora of neuroactive substances, which in some cases have opposing actions on the targeted SON/PVN neurons. Thus, a future challenge will be to elucidate more precisely how gliotransmitter release is precisely regulated.

The majority of the work reported so far on neuro-glial interactions in the SON/PVN has been centered on magnocellular neurosecretory neurons. However, given the high degree of neuronal heterogeneity within the PVN, and the importance of preautonomic neurons in neurohumoral integration, it will be critical to define whether the same principles reported in the magnocellular neurosecretory system applies to the other major neuronal systems in the PVN.

In the cortex and the hippocampus, brain regions that display a well-defined and highly organized laminar cytoarchitecture, astrocytes are also highly organized, comprising segregated spatial domains. Each domain represent a volume of neuropil under the control of a single astrocyte, and contains ~150,000 synapses (Bushong et al., 2002; Halassa et al., 2007; Ogata et al., 2002; Volterra et al., 2005). The PVN, on the other hand, displays a less-defined, and heterogeneously organized architecture. Thus, whether astrocytes in the PVN (and SON) also display organized and segregated domains, and whether an astrocytic volume domain is restricted to a specific neuronal type, is still unknown.

Finally, while most studies in the neuro-glial communication field have focused on astrocytes, microglial cells constitute another major glial cell type in the brain. Microglia cells, the resident immune cells of the brain (Saijo et al., 2011) contribute to a number of CNS homeostatic processes, including surveillance of their surroundings (Nimmerjahn et al., 2005), brain development and maturation (Dalmau et al., 1998; Fiske et al., 2000; Perry et al., 1985; Tremblay et al., 2011b; Wake et al., 2009), and synaptic remodeling (Tremblay et al., 2011a), among others. Moreover, microglia are the first responders to injury and pathogen infection in the brain, turning into an activated, pro-inflammatory state (Saijo et al., 2011). Active microglia release a variety of pro-inflammatory and also neuroactive substances, including cytokines, chemokines, reactive oxygen species, and nitric oxide, among others (Saijo et al., 2011), leading to gliosis, exacerbated neuronal activity, and ultimately, neuronal cell death. Importantly, there is a growing body of evidence supporting that cardiovascular-related signals, in particular angiotensin II, can lead to microglial activation (Joglar et al., 2009; Rodriguez-Pallares et al., 2008; Shi et al., 2010; Villar-Cheda et al., 2012), contributing in turn to exacerbated neurohumoral activation in disease conditions, such as heart failure, hypertension and diabetes (Francis et al., 2004; Luo et al., 2002; Rana et al., 2010; Shi et al., 2010). Thus, future studies are warranted to start elucidating communication modalities among microglial cells, neurons and astrocytes within CNS areas involved in autonomic and neuroendocrine regulation.

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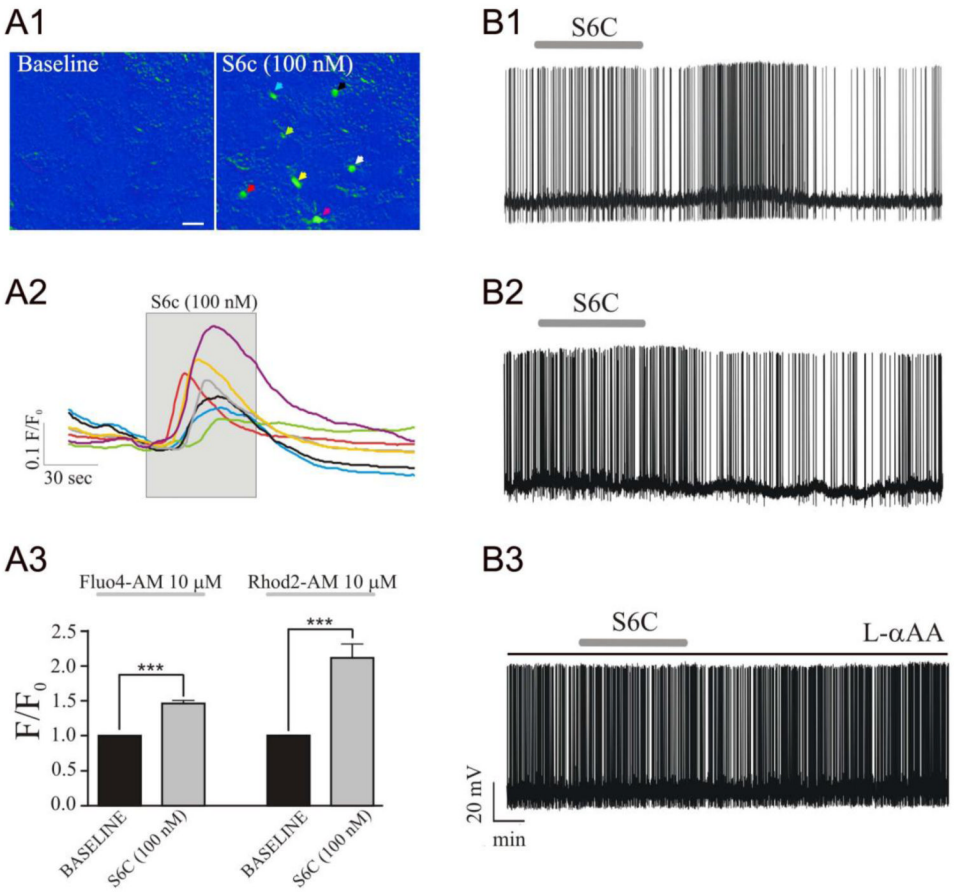

Figure 1. Endothelin B (ET_B) receptor activation in the SON increases intracellular Ca^{2+} levels in astrocytes, and evokes a delayed change in SON firing activity

A1, Representative pseudocolor images of transient Ca^{2+} changes in Fluo-4 loaded astrocytes (arrows) induced by the ET_B receptor agonist sarafotoxin (S6c). **A2**, traces of ΔCa^{2+} ($\Delta F/F_0$) corresponding to the astrocytes shown in A1. **A3**, Summary data of $\Delta F/F_0$ in response to S6c in astrocytes loaded with Fluo4-AM or Rhod2-AM. **B**, Bath application of Sarafotoxin 6c (S6c, 100 nM) induced either excitatory (**B1**) or inhibitory (**B2**) responses in SON neurons. Pre-incubation of hypothalamic slices in the presence of the gliotoxin L-αAA (250 µM) prevented S6c effects on SON firing activity (**B3**). ***P< 0.001. Scale bar= 10 µm. Modified from (Filosa et al., 2012).

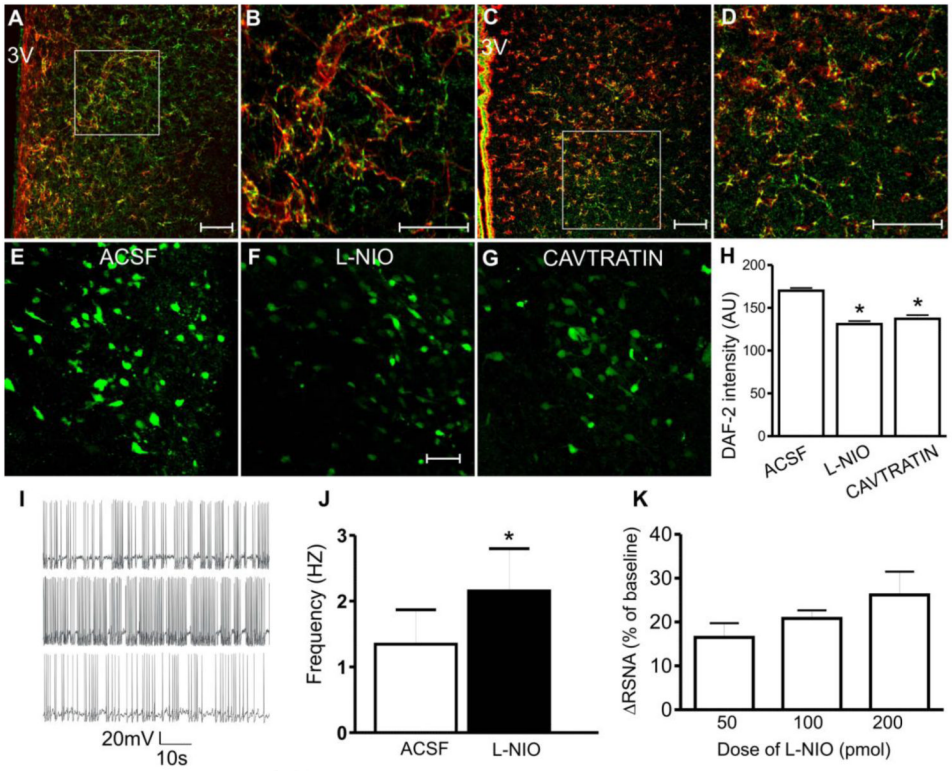

Figure 2. Glial eNOS modulates presympathetic neuronal activity and renal sympathetic nerve outflow in the PVN

A and **B**: Immunostaining of eNOS (green) and the glial marker GFAP (red) within the PVN. **B**: Higher magnification image of the area outlined in **A**. **C** and **D**: eNOS (green) and glial S100b (red) immunoreactivities within the PVN. **D**: Higher magnification image of the area outlined by the square in **C**. Note the dense eNOS staining in the PVN, and the colocalization (yellow color: green + red) with both glial markers. **E** – **G**: Photomicrographs showing staining for the nitric oxide sensitive dye DAF-2 under (**E**) basal condition (ACSF), or in the presence of (**F**) eNOS inhibitor L-NIO (10μmol/L), and (**G**) eNOS inhibitor Cavtratin (10μmol/L). **H**: Summary data showing mean DAF-2 intensity in each experimental condition. **I**: *in vitro* electrophysiological recordings sowing firing activity of a PVN-RVLM projecting neuron before (top), during (middle) and after (bottom) bath application of L-NIO (10μmol/L). **J**: Summary data for mean firing frequency in PVN-RVLM neurons. **K**: Dose-dependent changes in renal sympathetic nerve activity (RSNA) in response to microinjections of L-NIO (50, 100, and 200pmol) into the PVN. Scale bars: 50μm. 3V: third ventricle; **P*<0.05 *vs* *ACSF (**H** and **J**). Modified from (Biancardi et al., 2011).

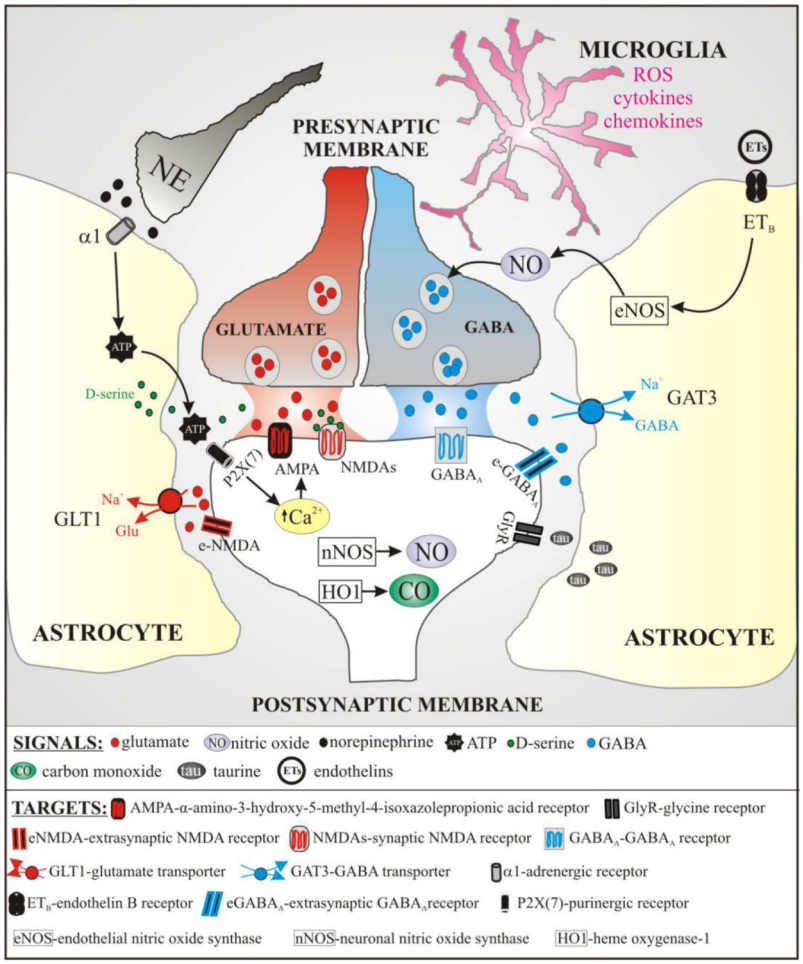

Figure 3. Schematic diagram depicting the various signals and targets at the SON/PVN tripartite synapse